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(71) Applicant *(for all designated States except US)*: ASTACAROTENE AB [SE/SE]; Idrottsvägen 4, S-134 40 Gustavsberg (SE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: LIGNELL, Åke [SE/SE]; Klippstigen 5, S-139 00 Värmdö (SE).

(74) Agents: ONN, Thorsten et al.; AB Stockholms Patentbyrå AB, Zacco & Bruhn (publ), P.O. Box 23101, S-104 35 Stockholm (SE).

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(54) Title: MEDICAMENT FOR IMPROVEMENT OF DURATION OF MUSCLE FUNCTION OR TREATMENT OF MUSCLE DISORDERS OR DISEASES

(57) Abstract

Medicament for the prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, e.g. equine Exertional Rhabdomyolysis, comprising at least one type of xanthophylles, e.g. astaxanthin, is described. Further, the use of xanthophylles in the preparation of such medicaments, and a method of prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, are disclosed.

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MEDICAMENT FOR IMPROVEMENT OF DURATION OF MUSCLE FUNCTION OR TREATMENT OF MUSCLE DISORDERS OR DISEASES.

The present invention relates to a medicament for the prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, comprising at least one type of xanthophylles, especially astaxanthin. The invention also relates to the use of at least one type of xanthophylles for the production of such a medicament and to a method of prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, e.g. equine Exertional Rhabdomyolysis.

Background of the invention

Exertional rhabdomyolysis, also referred to as exertional myopathy, tying-up syndrome, azoturia, or Monday morning disease, is probably the most common muscle disorder in horses. Predisposing or associated factors that have been implicated in the pathogenesis of this condition include electrolyte imbalances, hypothyroidism, and vitamin E-selenium deficiency. Therefore, treatment of horses affected by exertional rhabdomyolysis have included pain relief, rehydration and correction of electrolyte abnormalities (See e.g. The Horse: Diseases and Clinical Management, edited by C. N. Kolbluk, T. R. Ames, R. J. Geor, W.B. Saunders Company, Philadelphia, 1995, pp. 809-810).

Xanthophylles, including astaxanthin, is a large group of carotenoids containing oxygen in the molecule in addition to carbon and hydrogen. The carotenoids are

produced *de novo* by plants, fungi and some bacteria. Astaxanthin, in the form of naturally produced algal meal of cultured Haematococcus sp., has been marketed as antioxidant for mammals, especially humans.

Description of the invention

The present invention provides a medicament for the prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, comprising at least one type of xanthophylles.

In a preferred embodiment the type of xanthophyll is astaxanthin, particularly in a form esterified with fatty acids.

In a most preferred embodiment the astaxanthin in esterified form with fatty acids is algal meal of cultured Haematococcus sp.

Examples of mammalian muscle disorders or diseases include human myopaties and connective tissue diseases, as well as equine myopaties and connective tissue diseases.

In a particular embodiment of the invention, the mammalian muscle disorder is equine Exertional Rhabdomyolysis.

The medicament according to the invention may comprise a mixture of different types of xanthophylles or different forms of the same xanthophyll, such as a mixture of synthetic astaxanthin and naturally produced astaxanthin.

The medicament of the invention may comprise additional ingredients which are pharmacologically acceptable inactive or active in prophylactic and/or therapeutic use, such as flavoring agents, excipients, diluents, carriers, etc., and it may be presented in a separate unit dose or in admixture with food or feed. Examples of separate unit doses are tablets, gelatin capsules and predetermined amounts of

solutions, e. g. oil solutions, or emulsions, e.g. water-in- oil or oil-in-water emulsions. Examples of food in which the preparation of the invention may be incorporated is dairy products, such as joughurt, chocolate and cereals. The daily doses of the xanthophyll in the medicament of the invention will normally be in the range of 0.01 to 1 mg per kg body weight.

The present invention also comprises the use of at least one type of xanthophylles in the preparation of a medicament for the prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases. Once again, the preferred type of xanthophyll is astaxanthin, particularly in a form esterified with fatty acids, e.g. in the form of algal meal of cultured Haematococcus sp. ; and in a specific embodiment the mammalian muscle disorder is equine Exertional Rhabdomyolysis.

Further, the invention comprises a method of prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, e.g. equine Exertional Rhabdomyolysis, comprising administration to said mammal of a prophylactically and/or therapeutically effective dose of a medicament according to the invention.

Short description of the drawings

Figure 1 is a diagram showing the up-take of different carotenoids, e.g. astaxanthin, in rat muscle.

Figure 2 is a diagram showing the up-take of different carotenoids, e.g. astaxanthin, in rat heart.

Figure 3 is a diagram showing the carotenoid content in different rat organs after feed supplementation with astaxanthin.

Experiments

The medicament used in the experiments is the xanthophyll astaxanthin which was produced via culturing of the algae Haematococcus sp. by AstaCarotene AB, Gustavsberg, Sweden.

Astaxanthin from other sources, and other xanthophylles as well, are expected to be similarly useful for the purposes of the invention. An advantage of using astaxanthin from algae is, however, that the astaxanthin exists in a form esterified with fatty acids [Renström B. et al, 1981, Phytochem 20(11) :2561-2564], which esterified astaxanthin thereby is more stable during handling and storage than free astaxanthin.

Uptake of astaxanthin in rat

The experiment was conducted to establish if astaxanthin in the form of algal meal was taken up by rat and to see in which organs and tissues astaxanthin is accumulated.

Performance

A medicament in the form of feed containing 100 mg astaxanthin per kg feed in the form of algal meal was prepared.

Twenty-four male rats were divided into two groups; one group received feed without algal meal, and the other group received the feed containing algal meal.

After three weeks 6 rats from each group were sacrificed, and the remaining rats were sacrificed after 6 weeks.

At slaughter organs were excised, i. a. thigh muscle and heart, and they were freezed for later analysis of the content of carotenoids with the aid of HPLC.

Results

Astaxanthin could be demonstrated in both thigh muscle (see Fig. 1) and heart (see Fig. 2) of those rats that had received the feed supplemented with algal meal. In the control group, astaxanthin was not detectable.

Muscular tissue and particularly heart showed amongst the highest levels of astaxanthin after supplementation compared to the rest of the examined organs (see Fig. 3)

Effect of astaxanthin in horse

This preliminary experiment was conducted to establish if astaxanthin is taken up by horses and if supplementation with astaxanthin in the form of algal meal would improve the physical performance of trotting-horses.

Dosage

The horses received 100 mg astaxanthin per horse (approx. 500 kg) per day in the form of algal meal. The meal was supplied to the horses either sprinkled on concentrated feed or in the form of oil suspension.

Uptake

Astaxanthin could be demonstrated in muscles from horses that had received supplementation with the algal meal. The analyses were performed with the aid of HPLC on muscle biopsies. Astaxanthin could also be demonstrated in plasma samples from horses who had received the supplementation.

Effects

The most striking effect of the supplementation has been on horses suffering from muscle problems, so-called Exertional Rhabdomyolysis. In some horses this disorder appears when they are trained and raced regularly. It is not known what it is that causes the problems, but it is believed that the muscles are tightened and therefore the circulation is impaired, resulting in degradation of the muscular tissue. Today,

there is no remedy for the problem except rest and increased dosage of vitamin E in the feed.

Problem-horses who have received the astaxanthin-supplementation have been free from the symptom after 2 - 3 weeks, and they have been able to train and race in a normal way. In cases where the supplementation has been stopped or the dosage has been less than 30 mg astaxanthin per day, the septum has reoccurred after approximately 2 weeks. The algal meal supplement has been given to a total of 8 so-called problem-horses, and they have all responded positively to the supplementation.

Effect of astaxanthin on the physical performance of humans

The experiment was conducted so that for a period of 6 months, 20 healthy volunteers received 1 capsule containing 4 mg astaxanthin in the form of algal meal each morning in association with food, and 20 healthy volunteers received 1 capsule containing placebo.

Before the experiment was started, reference values were registered for each person with regard to strength/endurance, strength/explosiveness, condition, and weight.

Performance

The **strength/endurance** was estimated when a person made a maximum number of knee-bending in a Smith-machine with 40 kg load under standardized conditions.

The **strength/explosiveness** was tested under standardized conditions in a Wingate-machine with individually adapted load and registration of maximum effect during 30 seconds. The values were related to effect/ kg of body weight.

The **condition** was tested by a step test with 17 kg load and bench height of 32 cm until steady state pulse was reached. (I.e. the pulse did not differ more than three strokes from the measurement of the previous minute).

The **weight difference** between before and after the experiment was checked with a digital scale.

Results

No significant difference was established between the astaxanthin group and the placebo group in any of the tested parameters due to the small number of test persons.

With regard to condition (VO_2 max./kg, minute) there was no significant difference between the groups; a reduction of 1.75% for the astaxanthin group and 1.37% for the placebo group.

A reduction was also seen for both groups in the (strength/explosiveness) Wingate test (W/7 kg); - 4.13% for the astaxanthin group and - 5.81% for the placebo group.

Both groups gained weight ; 1.0% for the astaxanthin group and 2.1% for the placebo group. However, the individual differences were quite large, and no tendency could be established.

However, there was a clear difference between the groups in the strength/endurance test; 61.74% for the astaxanthin group and 23.78% for the placebo group.

In summary, the positive performance effect that was attributed to astaxanthin by individual athletes does not seem to be related to an increased condition or explosive strength but to strength/endurance according to this experiment.

CLAIMS

1. Medicament for the prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, comprising at least one type of xanthophylles.

2. Medicament according to claim 1, wherein the type of xanthophyll is astaxanthin.

3. Medicament according to claim 2, wherein the astaxanthin is in a form esterified with fatty acids.

4. Medicament according to claim 3, wherein the astaxanthin in esterified form with fatty acids is algal meal of cultured Haematococcus sp.

5. Medicament according to any one of claims 1 - 4, wherein the mammalian muscle disorder is equine Exertional Rhabdomyolysis.

6. Use of at least one type of xanthophylles in the preparation of a medicament for the prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases.

7. Use according to claim 6, wherein the type of xanthophyll is astaxanthin.

8. Use according to claim 7, wherein the astaxanthin is in a form esterified with fatty acids.

9. Use according to claim 8, wherein the astaxanthin in esterified form with fatty acids is algal meal of cultured Haematococcus sp.

10. Use according to any one of claims 1 - 9 , wherein the mammalian muscle disorder is equine Exertional Rhabdomyolysis.

11. Method of prophylactic and/or therapeutic improvement of the duration of mammalian muscle function and/or treatment of mammalian muscle disorders or diseases, comprising administration to said mammal of a prophylactically and/or therapeutically effective dose of a medicament according to any one of claims 1 - 4.

12. Method according to claim 9, wherein said mammalian muscle disorder is equine Exertional Rhabdomyolysis.

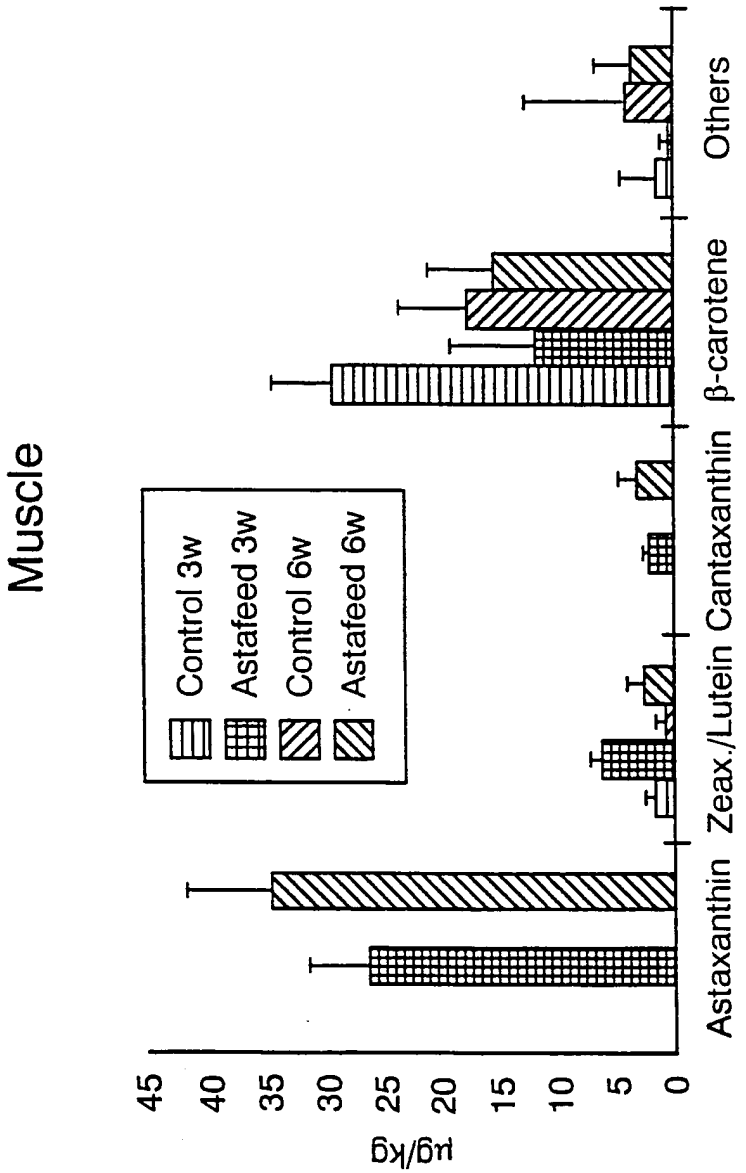
Muscle
Control 3w
Astafeed 3w
Control 6w
Astafeed 6w
µg/kg
45
40
35
30
25
20
15
10
5
0
Astaxanthin
Zeax./Lutein
Cantaxanthin
β-carotene
Others

FIG.1

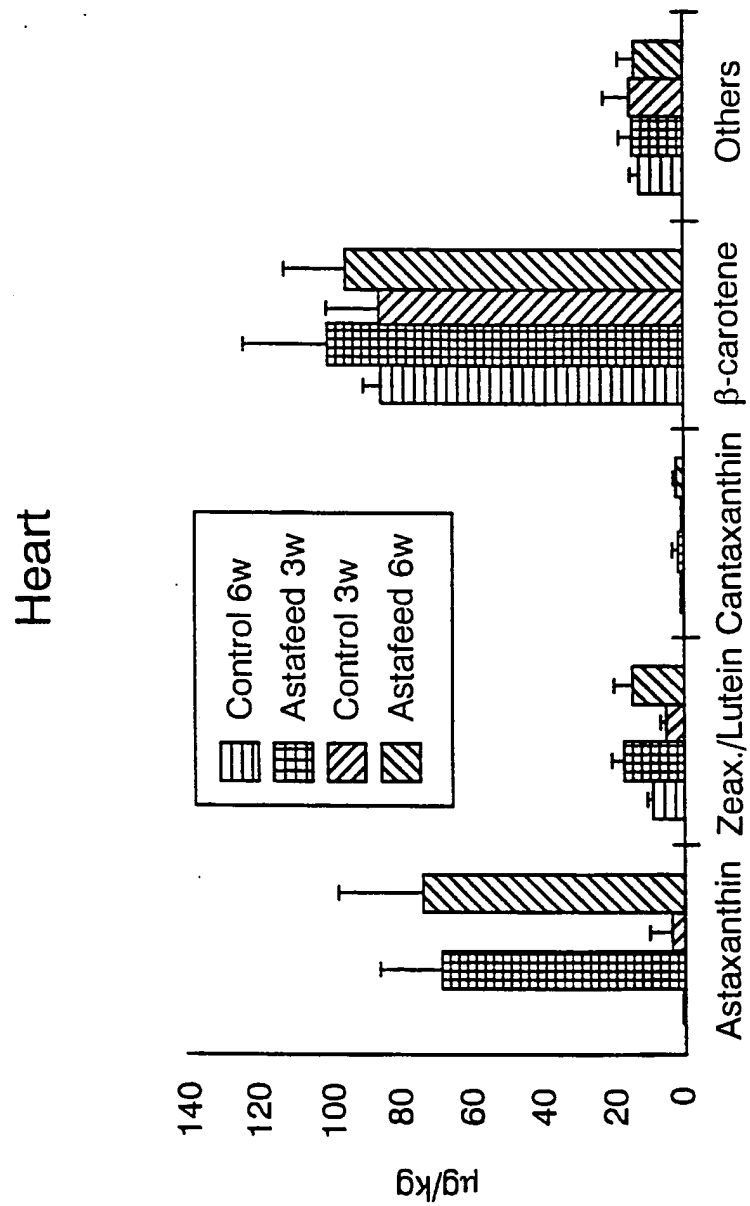
Heart
Control 6w
Astafeed 3w
Control 3w
Astafeed 6w
µg/kg
140
120
100
80
60
40
20
0
Astaxanthin
Zeax./Lutein
Cantaxanthin
β-carotene
Others

FIG.2

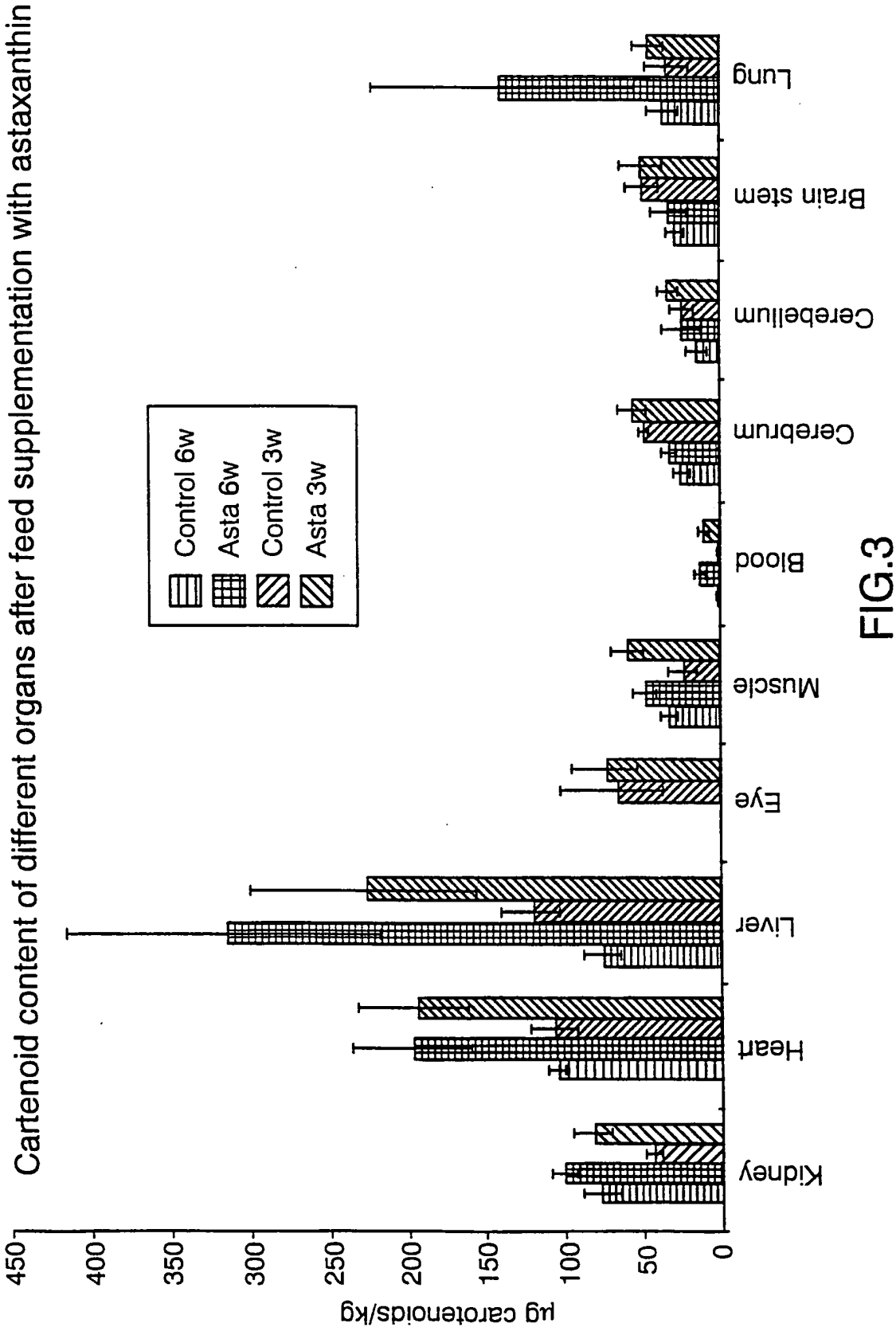
Cartenoid content of different organs after feed supplementation with astaxanthin
µg carotenoids/kg
0
50
100
150
200
250
300
350
400
450
Control 6w
Asta 6w
Control 3w
Asta 3w
Kidney
Heart
Liver
Eye
Muscle
Blood
Cerebrum
Cerebellum
Brain stem
Lung

FIG.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01526

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/12, A61K 31/07
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, WPI, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0770385 A1 (SUNTORY LIMITED), 2 May 1997 (02.05.97) --	1-12
X	Patent Abstracts of Japan, Vol 14,No 215, C-716 abstract of JP 2-49091 A (Suntory Ltd), 19 February 1990 (19.02.90) --	1-5
X	Patent Abstracts of Japan, Vol 18,No 307, C-1211 abstract of JP 6-65033 A (Lion Corp), 8 March 1994 (08.03.94) --	1-5

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 November 1998	01 -12- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Strandell Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01526

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9500130 A1 (THE HOWARD FOUNDATION), 5 January 1995 (05.01.95), page 7, line 15, the claims ---	1-5
X	WO 8503226 A1 (L'OREAL), 1 August 1985 (01.08.85), the claims ---	1-5
X	WO 9623489 A2 (BASF AKTIENGESELLSCHAFT), 8 August 1996 (08.08.96), page 3, line 14 - line 37, the claims --- --------	1-5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01526

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 11, 12
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 11, 12 relate to methods of treatment of the human or animal body by surgery or by therapy. see PCT, Rule 39.1(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/11/98

International application No.
PCT/SE 98/01526

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0770385 A1	02/05/97	AU 7040496 A	01/05/97
		JP 9124470 A	13/05/97
		SG 43432 A	17/10/97
WO 9500130 A1	05/01/95	AU 7005694 A	17/01/95
		GB 2280110 A,B	25/01/95
		GB 9412938 D	00/00/00
		IL 110139 D	00/00/00
		ZA 9404633 A	25/10/95
WO 8503226 A1	01/08/85	BE 901577 A	25/07/85
		BE 901578 A	25/07/85
		CH 666619 A,B	15/08/88
		CH 666620 A,B	15/08/88
		DE 3590001 T	15/05/86
		DE 3590002 T	15/05/86
		FR 2558372 A,B	26/07/85
		GB 2162748 A,B	12/02/86
		GB 2163051 A,B	19/02/86
		JP 61501030 T	22/05/86
		JP 61501031 T	22/05/86
		US 4931467 A	05/06/90
		WO 8503225 A	01/08/85
WO 9623489 A2	08/08/96	AU 4715796 A	21/08/96
		CA 2210957 A	08/08/96
		DE 19503604 A	08/08/96
		EP 0806946 A	19/11/97
		DE 19539743 A	30/04/97